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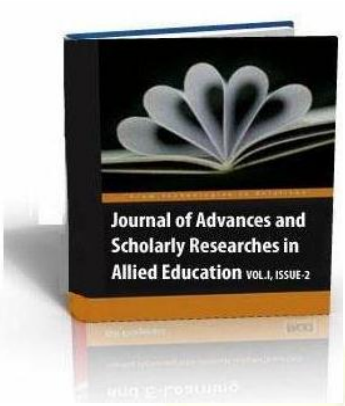
Identification of Host Range, Biophysical Properties and Electron Microscopic Investigation of the Virus Isolates in Pea



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ABSTRACT

Plant species of families Chenopodiaceae, Leguminosae and Solanaceae (only Petunia 63 hybrida) fall in host range of present virus isolate. Virus isolate found to infect the test plants namely Chenopodium album L., Chenopodium amaranticolor Coaste and Reyn., C. quinoa Wild, C. murale L. Pisum sativum L., Petunia hybrida Vilm, Vicia faba L. However, it could not infect Amaranthus caudatus L., Brassica campestris sp. rapa, Capsicum annuum L., Datura metel L., Datura metel L. var. festuosa, Nicotiana glutinosa L., Nicotiana tabacum L. var. Electron microscopic investigations through ISEM studies revealed that it had flexuous rod shaped particles with close affinity to PSbMV as it resulted in decoration of the virus particles with PSbMV antibodies.

INTRODUCTION

Pea (Pisum sativum L.) having chromosome number, 2n=14 belonging to family Fabaceae is an important legume crop grown worldwide mainly for its green pods which are consumed as fresh vegetable or in a cooked form. It has long been recognized as an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals. It contains high percentage of digestible protein (7.2 per cent) along with carbohydrate (15.9 per cent), vitamin A (405 IU) and 52 mg/100g vitamin C (Rai and Yadav, 2005). It is also a rich source of the major and minor minerals including potassium (1.04 per cent of dry weight) which is present in highest concentration, followed by phosphorous (0.39 per cent), magnesium (0.10 per cent) and calcium (0.08 per cent) besides seven trace minerals as 97 ppm iron, 42 ppm selenium, 41 ppm zinc, 12 ppm molybdenum, 11 ppm manganese, 9 ppm coppper and 4 ppm boron (Reichert and Mackenzie, 1982). Pea also contains a variety of phyto-chemicals including phenolic compounds, phytates, saponins and oxalates. The major phenolic constituents in pea are tannins, phenolic acids and flavonoids. Which has been recognized for their ability to act as antioxidants.

Most of phenolic compounds with the high concentrations are present in the seed coat, particularly in darkseeded varieties. Pea also contain other minor constituents which exhibit bioactivity and positive benefits on human health, including saponins and phytates, which exhibit hypocholesterolaemic and anticarcinogenic activities.

In India, it is grown as winter season vegetable in the Central and Northern plains and as a summer and autumn-winter crop in the hilly regions. Major pea producing states are Uttar Pradesh, Bihar, Haryana, Himachal Pradesh, Orrisa and

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Karnataka. It occupies an area of about 433.6 thousand hectares with annual production of 3868.6 thousand metric tonnes (Anonymous, 2015). It is one of the most popular off season vegetable crop grown in north-western Himalayan region of India (Sharma et al., 2013).

REVIEW OF LITERATURE

Peas are susceptible to a large number of aphid- transmitted viruses, which can produce diseases individually or in combination. Many common names have been used to describe these diseases, and to avoid confusion they will be mentioned in the discussion under each virus. The main viruses infecting pea belong to three distinct virus groups designated by virologists. Pea enation mosaic is the only member of one group, pea streak and red clover vein mosaic belong to a second group, and the final group includes bean yellow mosaic (also called pea mosaic), clover yellow vein, and pea seedborne mosaic.

Pea enation mosaic virus (PEMV) is one of only a few viruses with unique properties and hence has been assigned to a separate virus group. The virus mainly infects legumes in the temperate regions of the world. In addition to pea, PEMV also infects broadbean (from which the virus was first described in New York in 1935), sweet pea, and alfalfa and probably overwinters in many common perennial legumes. The virus is spread in nature most efficiently by the pea aphid *(Acyrthosiphon pisum)* and to a lesser extent by the green peach aphid *(Myzus persicae).* The virus is transmitted in a persistent (circulative) manner.

MATERIAL AND METHODS

COLLECTION AND MAINTENANCE OF VIRUS ISOLATES

The cultures of mosaic isolates were collected from three different pea growing localities of Shahjahanpur. The isolates collected on the basis of symptoms and localities were maintained on healthy seedlings of pea variety “PB-89” by mechanical sap inoculation under insect proof glasshouse conditions.

RAISING AND MAINTENANCE OF TEST PLANTS

Healthy seedlings of pea and other test plant species were raised in earthen pots filled with sterilized soil and FYM mixture (3:1 ratio v/v). Generally fifteen to thirty days old seedlings of the test plants at 2-3 leaf stage were used for inoculation. The pots containing test plants were maintained under insect proof glasshouse where the temperature varied between 25-35oC during experimentation.

PREPARATION OF INOCULUM

The inoculum of each isolate was prepared from the young leaves of infected plants showing pronounced symptoms. Leaves were harvested and washed thoroughly first with tap water and then washed with distilled water to remove the extraneous material. Moisture of leaves was removed by keeping them between the folds of blotting papers. The dried leaves were triturated in phosphate buffer (0.1 M pH 7.2) in a sterilized pestle and mortar. The slurry thus obtained was strained through two layers of muslin cloth in sterilized Petri plate. The standard extract thus prepared was used for mechanical inoculations.

HOST RANGE

To determine the host range of virus isolate, 17 plant species belonging to five different families: Amaranthaceae, Brassicacaea, Chenopodiaceae, Leguminosae and Solanaceae were raised under insect proof glasshouse conditions. Ten plants of each species were inoculated mechanically with the standard extract of virus isolate. Inoculated plants were observed for 4-6 weeks for the appearance of symptoms. The plants of different families were inoculated at 4-5 leaf stage. The plants which did not show any symptoms were back inoculated onto the original host (pea var. PB-89) to check the symptomless infections.

BIOPHYSICAL PROPERTIES

Biophysical properties of the isolate under investigations such as thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) were determined as per standard methods described by Noordam (1973).

Thermal inactivation point

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For determining the thermal inactivation point (TIP), standard extracts were prepared in 0.1 M phosphate buffer (pH 7.2). Two ml of the standard extract was pipette out in each thin walled test tube which were then subjected to different temperature ranging from 45-65oC in thermostatically controlled water bath. The test tubes were kept immersed in hot water bath at a particular temperature for 10 min for each treatment and were continuously shaken during the heating treatment. Heated tubes were then placed in ice cold water. The inoculum thus obtained was inoculated on test plants starting from the highest temperature, coming down to the lowest one. Non treated extract served as control.

Dilution end point (DEP)

Different dilutions of inoculum were prepared in test tubes by adding calculated amount of 0.1 M phosphate buffer (pH 7.2) to crude extract. The dilutions tested were 1:1, 1:10, 1:100, 1:1,000, 1:10,000, 1:1, 00,000 and 1:10, 00,000. The diluted sap was inoculated on the test plants starting with the highest dilution to lowest ones. Undiluted crude sap served as control.

Longevity in vitro (LIV)

The reaction of the virus infectivity in vitro was measured by storing the standard extract of the virus isolates at room temperature (22-26oC) and also at 4±1oC under refrigeration. The first inoculation was done immediately after preparation of the extract followed by inoculations at different intervals of storage. Sap extracted from healthy leaves and similarly kept at room and refrigeration temperature served as control.

ELECTRON MICROSCOPY

The particle morphology of virus isolate under study was determined by the following modifications of Brandes (1957) leaf dip preparation method.

ISEM (Immunosorbent electron microscopy: Clumping)

A drop of clarified crude virus extract was mixed with a drop of diluted (1:200) antiserum (in normal saline) on parafilm coated glass slide. It was then incubated at 37°C for one hour. Then collodion coated copper grid was placed, film side down, on the drop. It was again incubated at 37°C for one hour. Then the grid was washed with 30 drops of phosphate buffer, drained by touching edge with filter paper and then stained with 2 per cent aqueous uranyl acetate solution. Then the grid was finally drained and air dried and examined with transmission electron microscope.

RESULTS

SYMPTOMATOLOGY

The pea (var. PB-89) plants were inoculated mechanically at first true leaf stage by usual leaf rub method using the infectious sap extracted from a diseased pea plant. Visible symptoms on the leaves of inoculated pea plants were noticed in the form of vein clearing and mild mosaic within 15-20 days of inoculation under glasshouse conditions. Later mosaic, typical transient clearing and swelling of vein symptoms were developed on the leaves which turned into severe mosaic with the advancement of infection. As the infection progressed, stunting of plant with shortening and downward rolling of the leaflets were observed and growth of leaf lamina was also impaired. The infected plants also exhibited curling of tendrils along with rosetting of apical portion of plant. Severely infected plants either fail to bear pods or give rise to small distorted pods as compared to healthy plants. Different symptoms observed on the inoculated plants of pea are shown in Plate-3A&3B.

HOST RANGE

Results of the host range experiments are tabulated in revealed that the virus isolate could infect the plant species of the families Chenopodiaceae, Leguminosae and Solanaceae. The isolate could not infect the test plants belonging to the family Amaranthaceae and Brassicacaea.

Amongst the tested plant species, virus isolate produced typical symptoms of virus infection on Chenopodium amaranticolor Coaste and Reyn., C. quinoa Wild, Chenopodium album L., C. murale L., Pisum sativum L., Petunia hybrida Vilm, Vicia faba L., species. However virus isolate was not able to infect Amaranthus caudatus L., Brassica campestris spp. rapa, Capsicum annuum L. (chilli), Datura metel L., Datura stramonium L. var. festuosa, Nicotiana glutinosa L., N. tabacum L. var. White Burley, N. debneyi Dowin., Nicotiana occidentalis 37B and Raphanus sativus.

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Symptoms produced on the inoculated plants of some species in the host range of virus isolate are shown in differen.

BIOPHYSICAL PROPERTIES

The biophysical properties such as thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) of the virus isolate were determined according to the standard procedures described in “Materials and Methods”. The results thus obtained are produced here as under.

Thermal inactivation point (TIP)

The data presented in Table 4.7 revealed that the thermal inactivation point (TIP) of virus isolate lied between 55-60oC as evident from complete inactivation of the virus in the standard extract treated at 60oC for ten minutes which had lost the infectivity and as such no transmission of the virus took place on to healthy pea plants.

Dilution end point (DEP)

The dilution end point of the virus was determined by inoculation of the test plants with serial dilutions of the infectious sap. The percent transmission data of the experiment are presented in Table 4.8. It is evident from the data that the diluted infectious sap of virus isolate upto 1:1,000 was infective and resulted in sap transmission of the virus. The infectious sap of the virus isolate at a dilution of 1:10,000 lost the infectivity which resulted in non-transmission of the virus to test plants. Hence, the DEP of virus isolate was found to lie between 1:1,000- 1:10,000.

Longevity in vitro (LIV)

The longevity in vitro of the isolate was determined by keeping the infectious sap at room temperature (22-26oC) and under refrigeration (4+1 oC) as per the procedure given under “Materials and Methods”. The percent transmission data of the experiment are presented in Table 4.9.1 & 4.9.2. The data presented in Table 4.9.1 revealed that infectivity of the virus decreased with increase in the storage period. The virus isolate could retain its infectivity for 1 day only under room temperature (22-26°C).

The data set out in Table 4.9.2 indicate that the virus isolate had lost its infectivity when infectious sap was stored at freezing temperature (4+1 oC) with in one day of storage. Thus, the virus isolate is retain its infectivity for one day under refrigerated conditions.

CONCLUSION

Host range studies concluded that the present isolate of PSbMV has a narrow host range infecting plant species from the families of Chenopodiaceae, Leguminosae and Solanaceae. Studies on the biophysical properties revealed that the present virus isolate had TIP of 55-60°C, DEP of 10-3-10-4 and LIV of 1 day both at room temperature (22-26°C) and under refrigeration (4+1 oC). the isolate studied in the present investigations revealed its serological identity with PSbMV.

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